

**AMENDMENTS TO THE SPECIFICATION:**

**Please replace the first paragraph on page 1, lines 9-11 of the Specification with the following amended paragraph:**

The present invention relates to a laser scan type fluorescence microscope used for a fluorescence observation or a ~~eo-fœus point~~ confocal fluorescence observation in ~~application of an applications such as function~~ elucidation of the function or an imaging of a cell and the like cells.

**Please replace the paragraphs on page 1, line 19 through page 5, line 26 with the following amended paragraphs:**

[0003]

Fig. 1 is an outline block diagram showing one conventional example of a laser scan type ~~eo-fœal point~~ confocal fluorescence microscope.

The laser scan type ~~eo-fœal point~~ confocal fluorescence microscope of Fig. 1 comprises a laser light source section 51, an objective ~~lens~~ optical system 53 which condenses excitation light from a laser light source section 51 on a sample 52, a scanning means 54 which ~~makes scans a surface of the sample 52 with the excitation light from the laser light source section 51~~ scans on a surface of the sample 52, a pupil projection lens 55 arranged between the scanning means 54 and the objective ~~lens~~ optical system 53, a detection optical system 56 for detecting fluorescence which ~~is emanated~~ emanates from the sample 52 and ~~has penetrated~~ passes the objective ~~lens~~ optical system 53 and the pupil projection lens 55.

The laser light source section 51 has a laser light source 51a and, a collimating optical system ~~which consists of~~ including lenses 51b and 51d and a pinhole 51c, and a dichroic mirror 51e.

The objective ~~lens~~ optical system 53 has an objective lens 53a and an image forming lens 53b for forming an intermediate image of ~~an objective lens 53a and~~ the sample 52. Moreover, a ~~backside~~ back focal position of the objective lens 53a is ~~constituted so that it may become~~ made conjugate at with a position near the scanning means 54 by the image forming lens 53b and the pupil projection lens 55.

The scanning means 54 ~~consists of~~ is configured as a proxy type Galvano mirror having Galvano mirrors 54a and 54b.

The detection optical system 56 has a dichroic mirror 56a, a barrier filter 56b, a lens 56c, and a ~~eo-focal point~~ confocal pinhole 56d and a light receiving optical sensor 56e, such as a photomultiplier ~~and the like~~.

Furthermore, the microscope of Fig. 1 has a dichroic mirror 57 which leads the fluorescence from the sample 52 to the detection means 56 while leading the excitation light from the light source section 51 to the sample 52, a mirror 59 which deflects the light ~~which~~ transmitted through the pupil projection lens 55 to the image forming lens 53b, an ~~eye piece~~ eyepiece optical system 60 for observing the image of the sample 52, and a fluorescence lighting optical system 61 used ~~at the time of a~~ in normal fluorescence observation.

[0004]

Thus, in the laser scan type ~~eo-focal point~~ confocal fluorescence microscope as constituted in Fig. 1, the excitation light ~~emanated~~ emanating from the laser light source 51a is condensed at the pinhole 51c by the lens 51b, and then is converted into a beam of parallel light rays by the lens 51d. Then, this excitation light is led to the proxy type Galvano mirror section ~~that, which~~ is the scanning means 54 ~~through, via~~ dichroic mirrors 51e and 57, and the luminous flux beam of rays of it is shifted ~~to direction of two dimensions dimensionally in reference to the optical axis~~ by each rotation of Galvano mirrors 54a and 54b ~~to an optical axis, and it is condensed, to be formed as a primary image as being focused on the intermediate image position 58 through the pupil projection lens 55, and thus an primary image is formed~~. The excitation light condensed ~~to~~ at the intermediate image position 58 is irradiated to incident on the sample 52 in ~~at~~ a minute spot ~~like shape through~~ via the mirror 59, the image forming lens 53b, and the objective lens 53a. At this time, the surface of the sample 52 is scanned with the excitation light irradiated by the surface 52 of the sample is scanned by the scanning means 54.

[0005]

The backside back focal position of the objective lens 53a is projected by the image forming lens 53b and the pupil projection lens 55 near the proxy type Galvano mirror which is the scanning means 54.

Fluorescence excited on the sample 52 by irradiating irradiation with the excitation light, is led to the detection optical system 56 ~~through via~~ the objective lens 53a, the image forming lens 53b, the pupil projection lens 55, the scanning means 54, and the dichroic mirror 57. Then, a wavelength separation is carried out by the dichroic mirror 56a, and only the fluorescence ~~which transmitted that passes through~~ the eo-focal point confocal pinhole

through via the barrier filter 56b and the lens 56c is detected by the light receiving optical sensor 56e, such as a photomultiplier.

[0006]

In carrying out a normal fluorescence observation through the ~~eye~~ eyepiece optical system 60, a fluorescence lighting optical system 61 equipped with a different light source 61a from the laser light source 51a is used. Excitation light emanated emanating from the light source 61a is transmitted through a lens 61b and a filter 61c, and is reflected by the ~~diehroic mirror dichroic mirror~~ 61d, and illuminates the sample 52 through the ~~objective lens~~ objective lens 53a. Fluorescence excited on the sample 52 by irradiating irradiation with the excitation light is condensed by the ~~objective lens~~ objective lens 53a, and is subjected to wavelength separation is carried out by the ~~diehroic mirror dichroic mirror~~ 61d arranged at in the fluorescence lighting optical system 61, and it is observed through via the prism 60a of the ~~eyepiece optical system 60~~, and the eyepiece 60b of the eyepiece optical system 60 through the barrier filter 61e.

#### DISCLOSURE OF THE INVENTION

[0007]

Such a conventional laser-scan-type ~~eo-focal point~~ confocal fluorescence microscope is excellent in resolution, and it has an advantage that light from other than a minute spot to be observed can be eliminated. Thus, it is useful for carrying out an intracellular functional elucidation etc.

However, in the laser scan type ~~eo-focal point~~ confocal fluorescence microscope, the equipment itself becomes large since it is necessary to add an optical system such as a pupil projection lens 55 and a ~~scanning means~~ scanning means 54 mentioned above etc., in addition to an optical system used for a normal fluorescence observation, such as an objective lens 53a and an image forming lens 53b.

[0008]

That is, generally as for the optical system of a laser-scan type ~~eo-focal point~~ confocal fluorescence microscope, the focal length of an image forming lens ~~has become~~ is such long such as around 180mm. For this reason Consequently, a total length from a sample to the scanning means arranged near a conjugate position of a pupil of an objective lens becomes 400-500mm, and to enlarge the whole equipment ~~becomes enlarged~~.

[0009]

For this reason, a ~~eo-focal point~~ confocal fluorescence observation and a fluorescence observation become possible only in case that the sample is arranged on a stage of a microscope.

Moreover, when a ~~eo-focal point~~ confocal fluorescence observation is actually performed to a rat, a small animal or a cell under a cultivation environment where it is alive (in vivo), there is a restriction that the observation environment must be built on the stage. Furthermore, a laser scan type ~~eo-focal point~~ confocal fluorescence microscope is generally ~~constituted so that it may observe~~ designed to perform observation in a state where the optical axis of an objective lens becomes perpendicular to a surface of the stage. Therefore, it is difficult to observe the sample from a slant direction. Moreover, it is difficult to ~~observe by~~ perform observation upon leaning the whole laser scan type ~~eo-focal point~~ confocal fluorescence microscope to the sample, or ~~to observe by~~ leaning the sample and the stage.

#### BRIEF SUMMARY OF THE INVENTION

[0010]

The present invention is made in view of problems mentioned above, and it aims at providing a laser scan type fluorescence microscope, ~~which can be miniaturized~~ that is small in size compared with the conventional laser scan type ~~eo-focal point~~ confocal fluorescence microscope, and that can observe achieve observation in a state that where a cell is alive (in vive vivo) by with wavelengths from a visible region to a near-infrared region with sufficient user-friendly operation.

[0011]

In order to attain the above-mentioned purpose, the laser scan type fluorescence microscope according to the present invention comprises a laser light source section, an objective ~~lens~~ optical system which condenses excitation light from the laser light source section on a sample, a scanning means which makes the excitation light from the laser light source section scan on a surface of the sample, a pupil projection lens arranged between the scanning means 54 and the objective ~~lens~~ optical system, and a detection optical system for detecting fluorescence which ~~is emanated~~ emanates from the sample and ~~has penetrated~~ passes the objective ~~lens~~ optical system and the pupil projection lens. The objective ~~lens~~ optical system has an objective lens, and an image forming lens for forming an intermediate image of the sample, and a ~~backside~~ back focal position of the objective lens ~~constituted so that it may become~~ is made conjugate at with a position near the scanning means by the image forming lens and the pupil projection lens, and the following condition (1) is satisfied:

$$0.15 \leq D/L \leq 0.5 \quad \dots(1)$$

where D is a ~~eo-focal length~~ parfocal distance of the objective lens, and L is a distance from the sample surface to the ~~conjugate~~ position of conjugate with the backside back focal position of the objective lens and arranged near the scanning means.

**Please replace the paragraph on page 6, lines 4-13 with the following amended paragraph:**

[0013]

In the laser scan type fluorescence microscope according to the present invention, the pupil projection lens ~~consists of~~ includes two or more lens groups, wherein a ~~concave~~ surface of a lens surface arranged at the nearest side of the scanning means is ~~directed~~ concave toward the scanning-means side, and a ~~concave~~ surface of a lens surface arranged at an nearest the intermediate image side is ~~directed~~ concave toward the intermediate image side, and the following condition (2) is satisfied;:

$$0.2 \leq F_e/D_3 \leq 0.5 \quad \dots(2)$$

where D<sub>3</sub> is a distance from the ~~conjugate~~ position of that is conjugate with the pupil of the objective lens and is located near the scanning means to the intermediate image position of the image forming lens, and F<sub>e</sub> is a focal length of the pupil projection lens.

**Please replace the paragraphs on page 6, line 25 through page 7, line 22 with the following amended paragraphs:**

[0015]

The laser scan type fluorescence microscope according to the present invention is characterized in that the image forming lens ~~consists of~~ includes two lens groups having that are a front group ~~at the side of an~~ arranged on the intermediate image side and a rear group ~~at the side of an~~ arranged on the objective lens side, ~~and the lens group of~~ the front group of the image forming lens has at least one negative lens, and the following conditions (5) and (6) are satisfied;:

$$0.4 \leq D_2/FTL \leq 1 \quad \dots(5)$$

$$0.7 \leq FTL_1/FTL \leq 1.5 \quad \dots(6)$$

where FTL<sub>1</sub> is a focal length of the rear group of the image forming lens, and D<sub>2</sub> is an interval between the front group of the image forming lens and the rear group of the image forming lens.

[0016]

The laser scan type fluorescence microscope according to the present invention comprises a first multi-mode fiber which leads the excitation light from the laser light source, section to the scanning means, a second multi-mode fiber which leads the fluorescence from the sample to the detection optical system, a first lens by which entry of the excitation light to the first multi-mode fiber is carried out, and a second ~~multi-mode fiber~~ lens by which leads entry of the fluorescence from the sample to the detection optical system is carried out, and the following conditions (7) to (9) are satisfied;:

$$2 \leq \Phi_{em}/\Phi_{ex} \leq 12 \quad \dots(7)$$

$$0.61 \times (\gamma_{ex}/N_A N_{Aex}) < \Phi_{ex} \quad \dots(8)$$

$$0.61 \times (\lambda_{em}/N_A \lambda_{em}) < \Phi_{em} \quad \dots(9)$$

where  $\Phi_{ex}$  is a diameter of a core of the first multi-mode fiber,  $\Phi_{em}$  is a diameter of a core of the second multi-mode fiber,  $N_A ex$  is ~~an~~ a numerical aperture number by which where entry of the excitation light to the first multi-mode fiber by the first lens is carried out,  $\lambda_{ex}$  is a wavelength of the excitation wavelength light,  $N_A em$  is ~~an~~ a numerical aperture number by which where entry of the fluorescence to the second multi-mode fiber by the second lens is carried out, and  $\lambda_{em}$  is a wavelength of the fluorescence wavelength.

**Please replace the paragraphs on page 8, lines 4 through page 23, line 1 with the following amended paragraphs:**

[0019]

By arranging the optical transmission means between the laser optical system and the scanning means, a degree of freedom can be given to arrangement of the optical system ~~of~~ in the main body that is from the scanning means to the objective lens, and of the laser optical system, and a miniaturized optical system ~~of~~ in the main body suitable for observation in a state where the sample is alive (*in vivo*) can be provided.

[0020]

By arranging ~~such that~~ the pupil projection lens, which relays ~~parallel luminous flux a~~ beam of parallel rays deflected by the scanning means to an intermediate image position ~~has, to include~~ two or more lens groups, ~~a concave surface of~~ with a lens surface arranged at the nearest side ~~of~~ the scanning means ~~is directed being concave~~ toward the scanning means side, and ~~a concave surface of~~ a lens surface arranged at the nearest side ~~of~~ the intermediate image side ~~is directed being concave~~ toward the middle-intermediate image side, correction of ~~an~~ optical performance in the intermediate image can be carried out well.

By satisfying the condition (2), ~~shortening of a distance from the scanning means to the intermediate image position, and miniaturization of the equipment can be achieved with a shortened distance from the scanning means to the intermediate image position, while making an good optical performance of the pupil projection lens good is achieved.~~

[0021]

By satisfying the condition (3), ~~shortening of a distance from a shoulder of the object lens on a body to the intermediate image position, and miniaturization of the equipment can be achieved with a shortened distance from the objective lens shoulder on the main body to the intermediate image position.~~

By satisfying the condition (4), ~~correction of spherical aberration generated by shortening a focal length, and chromatic aberration on the axis, which are caused by a shortened focal length, can be carried out well well compensated for.~~

[0022]

By constituting ~~that an the image forming lens consists of with two lens groups, correction of aberration in a first group compensation for aberrations~~ can be carried out by a the rear group so that ~~the aberration aberrations~~ produced in the front group ~~may be is~~ offset, and a suitable laser beam microscope is obtained by ~~that is suitable for observation at a state in vivo is achieved.~~

By satisfying the conditions (5) and (6), ~~correction of astigmatic astigmatism, coma aberration and magnification chromatic aberration can be carried out well compensated for.~~

[0023]

In a constitution comprising a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, and a second multi-mode fiber which leads fluorescence from the sample to the detection optical system, by satisfying the conditions (7) to (9), the ~~rate proportion~~ of the amount of ~~detected~~ fluorescence ~~detected~~ to the amount of excitation light from the light source becomes high, ~~and so that~~ brighter fluorescence ~~can be is detected, and furthermore. Furthermore, picture information of a on~~ the thickness direction from the sample can be obtained covering a predetermined thickness. Accordingly, operation performance in observation in the state ~~that where~~ the sample is alive (in vivo) is improved.

[0024]

By arranging the optical transmission means between the pupil projecting lens and the detection optical system, a degree of freedom can be given to arrangement of the optical system ~~of in~~ the main body that is from the pupil projecting lens to the objective lens, and of

the detection optical system, and a miniaturized optical system of the main body suitable for observation in a state (in vivo) of the sample can be constituted.

Further, if the optical transmission means which leads the excitation light from the laser light source section to the scanning means, and the optical transmission means which leads the fluorescence from the sample to the detection optical system are arranged by composed of separate optical fibers, respectively, the detection optical system can avoid an influence of the self-generated thereon by auto-fluorescence, which is generated when the excitation light enters into the optical fiber, and thus can detect the fluorescence generated by the sample can be detected with high precision accuracy.

#### BRIEF EXPLANATION OF DRAWINGS

#### BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWING

[0025]

Figure 1 is an outline block schematic configuration diagram showing one conventional example of a laser scan type ~~eo-focal point~~ confocal fluorescence microscope.

Figure 2 is an outline block a schematic configuration diagram of a first embodiment of a laser scan type fluorescence microscope according to the present invention.

Figure 3 is a principal-part diagram for explaining a principal part showing an outline a schematic configuration of the optical system arranged at in the main body portion in of the microscope of Fig. 2.

Figure 4 is a diagram showing an optical arrangement, a configuration in which a laser light source section and a detection optical system are added to the optical system of a in the main body portion of the microscope shown in Fig. 3.

Figure 5 is a diagram showing an outline optical arrangement a schematic configuration of an optical system in a second embodiment of the laser scan type fluorescence microscope according to the present invention.

Figure 6 is a diagram showing an outline optical arrangement a schematic configuration of an optical system in a third embodiment of the laser scan type fluorescence microscope according to the present invention.

Figure 7 is a diagram showing an outline optical arrangement a schematic configuration of an optical system in a fourth embodiment of the laser scan type fluorescence microscope according to the present invention.

Fig. 8 is a sectional diagram view taken along the optical axis for showing an optical arrangement developed along an optical axis of a pupil projection optical system and an

objective optical system and a concerning the of a first embodiment, used in the laser scan type fluorescence microscope of the present invention.

Fig.9 is a sectional diagram view taken along the optical axis for showing an optical arrangement developed along an optical axis of a pupil projection optical system and an object objective optical system of the third a second embodiment according to, used in the laser scan type fluorescence microscope of the present invention.

Fig.10 is a sectional diagram view taken along the optical axis for showing an optical arrangement developed along an optical axis of an a pupil projection optical system and an object objective optical system of the a third embodiment according to, used in the laser scan type fluorescence microscope of the present invention.,

#### BEST MODE FOR CARRYING OUT THE INVENTION

#### DETAILED DESCRIPTION OF THE INVENTION

[0026]

Figure 2 is an outline block a schematic configuration diagram of the first embodiment of a the laser scan type fluorescence microscope according to the present invention. Figure 3 is a principal-part diagram for explaining a principal part showing an outline a schematic configuration of an optical system arranged at in the main body portion in of the microscope of Fig. 2. Figure 4 is a diagram showing an optical arrangement, a configuration in which a laser light source section and a detection optical system are added to the optical system of a the main body portion of the microscope shown in Fig. 3.

The laser scanning fluorescence microscope of the first embodiment comprises, in a main body 11 of the microscope, a laser light source section 1, an exchangeable objective lens unit 2, a scanner section 3 as a scanning means, a lens unit 4 equipped with including a pupil projection lens 6 and an image forming lens unit 5, and a detection optical system 7 in a main body 11 of a microscope.

It is desirable to use a semiconductor laser in a the laser light source section 1 since a the main body portion 11 of the microscope can be miniaturized by using it.

The objective lens unit 2 is constituted by the objective lens optical system 8 forms, together with the image forming lens unit 5, an objective optical system 8. The objective lens optical system 8 has a function which condenses of condensing excitation light from the laser light source section 1 on a sample 10 on a stage 9. Moreover, the objective lens unit 2 is constituted configured so that a its backside focal position may become is made conjugate by

with a neighborhood position of near the scanner section 3 by the image forming lens unit 5 and a pupil projection lens 6. The image forming lens unit 5 has a function which forms of forming an intermediate image of the sample 10.

[0027]

The pupil projection lens 6 is arranged between the scanner section 3 and the objective lens optical system 8.

A detection optical system 7 has a barrier-filter 7a, a lens 7b, a ~~eo-focal point~~ confocal pinhole 7c, and a light receiving optical sensors sensor 7d, and it is constituted configured so that the fluorescence which is emanated emanating from the sample 10 and transmitted through the objective lens optical system 8 and the pupil projection lens 6 ~~may be~~ is detected by the light receiving optical sensor 7d.

The laser light source section 1 has a laser light source 1a and a collimating optical system, which consists of laser light source 1a, and Lenses that is composed of lenses 1b and 1d and a pinhole 1c.

Between the scanner section 3 and the detection optical system 7, a dichroic mirror 16 is arranged for leading fluorescence from the sample 10 to the detection means 7, while leading excitation light from the light source section 1 to the sample 10 is arranged.

A laser drive section 14 ~~that drives emission of laser light from the laser light source 1a~~ is connected to with the laser light source section 1 for driving emission of laser light from the laser light source 1a.

A focusing mechanism portion section 12 for focusing the objective lens unit 2 is arranged on the main body portion 11 of the microscope.

In addition, the laser scan type fluorescence microscope has a an x-y-θ main part body moving mechanism 13 for adjusting performing positional adjustment of the main body 11 of the microscope, in two dimensions and with respect to an angle θ of observation to a at which the specimen is observed and a position in directions of two dimensions with respect to the main body portion 11 of the microscope.

The laser scan type fluorescence microscope is connected with a processing control means 15, such as a personal computer and the like. The processing control means 15 is constituted constructed and arranged to carry out a wavelength control of laser light emanating from the laser light source emanated by driving as driven by the laser drive section 14; wavelength selection of the dichroic mirrors, the filters, etc.; control of a wavelength separation element; drive control of the laser drive section 14; analysis and display control of detection information received by the light receiving optical sensor 7d of the detection

optical system 7; drive control of the scanner section 3; drive control of a the focusing mechanism portion section 12; and; drive control of the x-y-θ main part body moving mechanism 13; and so on.

[0028]

In the laser scan type fluorescence microscope of the first embodiment, the excitation light emanated emanating from the laser light source 1a is condensed on the pinhole 1c by the lens 1b, and is converted into a beam of parallel light rays by the lens 1d. Then, it is led to the scanner section 3 through via the diachronic mirror 16, and luminous flux the beam of rays is shifted to two directions of two dimensions two-dimensionally in reference to the optical axis by each rotation of Galvano mirrors 3a and 3b of the scanner section 3 to the optical axis, and by condensing, to be condensed at the intermediate image position as a primary image through the pupil projection lens 6, image forming of the primary image is carried out. The excitation light, which is condensed at the intermediate image position, is irradiated by incident on the sample 10 through the image forming lens unit 5 and the objective lens unit 2 in a shape like a minute spot. At this time, the excitation light irradiated to the tenth, with which the surface of the sample scanned 10 is irradiated, is made to scan by the scanner section 3.

[0029]

The backside focal position of the objective lens unit 2 is projected near the scanner section 3 by the image forming lens unit 5 and the pupil projection lens 8.

The fluorescence excited on the sample 10 by irradiating irradiation with the excitation light is led to the detection optical system 7 through an via the objective lens unit 2, the image forming lens unit 5, the pupil projection lens 6, the scanner section 3, and the dichroic mirror 16. Then, only the fluorescence transmitted through the eo-foveal point confocal pinhole 7c via the barrier filter 7a and the lens 7b is detected by the light receiving optical sensor 7d, such as a photo multiplier.

[0030]

Here, the laser scan type fluorescence microscope of the first embodiment is constituted configured to satisfy the following condition;

$$0.15 \leq D/L \leq 0.5 \quad \dots(1)$$

where D is a eo-foveal length parfocal distance of the objective lens unit 2, and L is a distance from the surface of the sample 10 to the conjugate position E of conjugate with the backside back focal position of the objective lens unit 2 arranged and located near the scanning means (scanner section 3) from the tenth surface of the sample.

[0031]

When the condition (1) is satisfied as mentioned in the laser scan type fluorescence microscope of the first embodiment, it becomes possible to shorten a distance from the scanning means 3 to the sample 10, and miniaturization of the equipment can be achieved.

If it exceeds the ~~maximum value upper limit~~ of the condition (1), the focal lengths of the image forming lens 5 and the pupil projection lens 6 become short, and an interval between the pupil projection lens 6 and the scanning means 3 becomes short too much, and an interference occurs.

On the other hand, if it is less than the lower limit of the condition (1), the full length from the sample 10 to the scanning means 3 becomes long too much, and the miniaturization of the equipment becomes difficult.

[0032]

Figure 5 is a diagram showing ~~an outline optical arrangement a schematic configuration of an optical system in a~~ the second embodiment of the laser scan type fluorescence microscope according to the present invention. Here, the same symbol is ~~symbols are used to show a component components having the same composition as~~ in the first embodiment.

The laser scan type fluorescence microscope of the second embodiment is a modification of the first embodiment, where a dichroic mirror 20, a lens 19, an optical transmission means 18, a lens 17, and the dichroic mirror 16 are arranged between ~~the a~~ light source section + 1' and the scanning means 3.

~~An~~ The optical transmission means 18 ~~consists is composed~~ of an optical fiber, such as a single mode fiber or a multi-mode fiber. Since an end surface of the optical transmission means 18 is conjugate to ~~with~~ a specimen surface position and a core diameter of the end surface of the fiber serves as a ~~co-focal point~~ ~~confocal~~ pinhole, ~~the a~~ pinhole 1c of a light source section ~~light source section~~ 1' and ~~the a~~ pinhole 7c of a ~~second~~ detector 7' may be arranged off ~~removed from~~ an optical path; or ~~the diameter~~ may be big enough ~~have~~ sufficiently large diameters in reference to a ~~the~~ diffraction diameter. When ~~an~~ ~~the~~ optical transmission means 18 is a multi-mode fiber, a ~~co-focal point effect becomes weaker since an~~ ~~a fiber its large core diameter becomes large in reference to a~~ ~~the~~ diffraction ~~core diameter~~ ~~weakens the confocal effect, but it is makes it~~ possible to pick up a fluorescence image brightly. Therefore, it is ~~good~~ ~~preferred~~ to choose a fiber according to ~~an~~ ~~the~~ purpose of observation ~~purpose~~. When performing a non-confocal, normal fluorescence observation which is not a ~~co-focal point~~ by using a multi-mode fiber for ~~an~~ ~~the~~ optical transmission

means 18, it is ~~good constitute that preferred to remove~~ the pinhole 1c of the ~~light source section~~ light source section 1', and the pinhole 7c of the second detector 7' ~~are not arranged in from~~ the optical path.

[0033]

The dichroic mirror 20 is ~~constituted so that~~ configured to lead fluorescence from the sample 10 ~~may be led~~ to the second detection optical system 7', while leading the excitation light from the light source section 1' to the sample 10.

And ~~it is constituted the configuration is made so that, via the optical transmission means~~ 18, the fluorescence from the sample 10 ~~which transmitted~~ through the lens 17 is led to the second detection means 7 7', while excitation light from the laser light source section 1' is led to the scanning means 3 ~~through the optical transmission means~~ 18.

In ~~an~~ the embodiment of Fig. 4, as for the ~~light source section~~ light source section 1', ~~two or more the a~~ a plurality of sets each including a light source 1a to ~~the a~~ lens 1d are prepared, and ~~a dichroic mirror~~ dichroic mirrors 1e ~~is~~ are arranged accordingly.

[0034]

If an optical transmission means is arranged between the ~~laser light source section~~ laser light source section 1 and the scanning means 3 as shown in the laser scan type fluorescence microscope of the second embodiment, it becomes possible to give a degree of freedom to arrangement of ~~an~~ the optical system ~~of~~ in the main body ~~portion~~ of the equipment from the scanner section 3 to the objective lens ~~unit~~ 2 and the laser light source section 1'. For this reason, ~~an~~ the optical system ~~of a~~ in the main body ~~portion~~ of a the microscope can be miniaturized in a suitable size for observation in a state of a sample being alive (in vivo).

In the second embodiment, if a near-infrared Femto-second pulsed laser is used for the laser light source 1', it is possible to make observation becomes possible using the microscope as a fluorescence microscope of with multiphoton excitation.

In this case, it is ~~good enough that~~ preferred to use the detector 7 ~~is used~~ as a detector of the ~~for~~ fluorescence having generated by multiphoton excitation, ~~and a to appropriately select the spectrum characteristic of the dichroic mirrors 1e, 16, and 20 are selected, and to make~~ pinhole diameters of the pinholes 1c and 7c ~~are made~~ sufficiently larger enough than a diffraction ~~core, diameter or they are arranged off~~ remove the pinholes from the optical path.

[0035]

Fig. 6 is a diagram showing an outline composition of the optical system in the third embodiment of the laser scan type fluorescence microscope concerning the present invention.

Here, the same symbol is symbols are used to show a component components having the same composition as in the second embodiment.

The laser scan type fluorescence microscope of the third embodiment is a modification of the second embodiment. A, where a lens 22, the optical transmission means 21 which consists is composed of an optical fiber, such as a single mode fiber or a multi-mode fiber, and a lens 23 are arranged between a the dichroic mirror 16 and a the detection optical system 7'. And it is constituted the configuration is made so that, while the excitation light from light source section a light source section 1" is led to the sample 10 via the optical transmission means 18, the fluorescence from the sample 10 may be is led to the second detection optical system 7' via the optical transmission means 21. As shown in the second embodiment, since end surfaces of optical fibers of the optical transmission means 18 and 21 are conjugate to with a specimen surface position and, core diameters of the end surfaces of the fibers serve as a co-focal point pinhole confocal pinholes, the a pinhole 1c of a light source section the light source section 1" and the pinhole 7c of a the detector 7' may be arranged off removed from the optical path, or the diameter may be big enough have sufficiently large diameters in reference to the diffraction diameter. When the optical transmission means 18 and 21 are multi-mode fibers, a co-focal point effect becomes weaker since a fiber their large core diameter becomes large diameters in reference to a the diffraction core diameter weaken the confocal effect, but it is they make it possible to pick up a fluorescence image brightly. Therefore, it is good preferred to choose a fiber according to an the purpose of observation purpose. When carrying out a non-confocal, normal fluorescence observation which is not a co-focal point, by using a multi-mode fiber fibers for the optical transmission means 18 and 21, it is good to constitute that preferred to remove the pinhole 1c of the light source section light source section 1"; and the pinhole 7c of the detector 7' are not arranged in from the optical path.

It is desirable to constitute such way as mentioned above, because an the optical system of in the main part body of a the microscope equipment can be miniaturized much more.

As shown in the laser scan type fluorescence microscope of the third embodiment, if If an optical fiber constituting the optical transmission means 18, which leads the excitation light from light source section the light source section 1" to a the scanner section 3, and an optical fiber constituting the optical transmission means 21, which leads the fluorescence from a the sample 10 through the pupil projection lens 6 to the second detection optical system 7', are separately arranged, respectively, the second detection optical system 7' can

avoid an influence of the self-generated thereon by auto-fluorescence, which is generated when the excitation light enters into the optical fiber 18, and thus can detect the fluorescence generated by the sample 10 can be detected with high precision accuracy.

In the laser scan type fluorescence microscope of the third embodiment, when the optical transmission means 18 and 21 are constituted by a composed of multi-mode fiber fibers, it is desirable to satisfy the following conditions (7) to (9).

$$2 \leq \Phi_{em}/\Phi_{ex} \leq 12 \quad \dots(7)$$

$$0.61 \times (\lambda_{ex}/NA_{ex}) < \Phi_{ex} \quad \dots(8)$$

$$0.61 \times (\lambda_{em}/NA_{em}) < \Phi_{em} \quad \dots(9)$$

where  $\Phi_{ex}$  is a core diameter of the multi-mode fiber 18,  $\Phi_{em}$  is a core diameter of the multi-mode fiber 21,  $NA_{ex}$  is an a numerical aperture size of incidence where light to is incident on the multi-mode fiber 18 by via the lens 19,  $\lambda_{ex}$  is an excitation wavelength,  $NA_{em}$  is an a numerical aperture size of incidence where light to is incident on the multi-mode fiber 21 by via the lens 22, and  $\lambda_{em}$  is a fluorescence wavelength.

[0037]

If the conditions (7) to (9) are satisfied, the rate proportion of the amount of detected fluorescence detected to the amount of excitation light from the light source becomes high, and possible to pick up so that a bright fluorescence image brightly, and to obtain is obtained. In addition, since a predetermined amount of picture information from the sample with respect to thickness direction. Therefore can be obtained, operation performance is improved in observation in the state that where the sample is alive (in vivo).

If it is less than the lower limit of the condition (7), since the rate proportion of the detected fluorescence detected to the excitation light from the light source becomes low, and the acquired fluorescence image obtained becomes dark, is darkened and the picture information of on the thickness direction of the sample extremely decreases very much. Therefore, the operation performance at the time of in observation worsens.

On the other hand, if it exceeds the maximum value upper limit of the condition (7), the too much picture information covering the thickness direction of the sample enters too much is obtained, and a picture other than the fluorescence to be observed can be seen too much becomes too conspicuous. Therefore, it becomes difficult to carry out a fluorescence observation.

If conditions (8) and (9) are not satisfied, the excitation light to the sample becomes weak, or the fluorescence intensity of detected fluorescence is dark and becomes low, to extremely decreases the picture information of on the thickness direction of the sample

decreases very much. Therefore the operation performance at the time of in observation worsens.

It is more desirable if the following condition (7-1) is satisfied.

$$4 \leq \Phi_{\text{em}}/\Phi_{\text{ex}} \leq 10 \quad \dots(7-1)$$

~~the~~ The composition of the laser scan type fluorescence microscope which satisfies the conditions (7) to (9) mentioned above of the third embodiment is applicable also to a general laser scan type fluorescence microscope which does not satisfy the condition (1).

[0038]

Fig. 7 is a diagram showing an outline composition of the optical system in the fourth embodiment of the laser scan type fluorescence microscope according to the present invention. Here, the same symbol is used to show a component having the same composition in the first embodiment.

The laser scan type fluorescence microscope of the fourth embodiment is a modification of the first embodiment, where a ~~light source section~~ light source section 1 consists is composed of a laser light source 1a and a lens 1d. The lens 22, the optical transmission means 21, the lens 23, and the detection optical system 7' are arranged at an opposite side of a Galvano mirror 3b which is separated from the dichroic mirror 16.

The optical transmission means 21 consists is composed of an optical fiber, such as a single mode fiber or a multi-mode fiber. An optical fiber end surface of the optical transmission means 21 is conjugate to with a specimen surface position, and since a core diameter of a ~~the~~ fiber end surface serves as a ~~co-focal point~~ confocal pinhole, the pinhole 1c' of the light source section 1 and the pinhole 7c of the detector 7' may be arranged off removed from the optical path, or may have a larger diameter to a in reference to the diffraction diameter. If the optical transmission means 21 is a multi-mode fiber, it is possible to pick up fluorescence image brightly, although a ~~co-focal point~~ the confocal effect becomes weaker as a fiber core diameter becomes large in reference to a ~~the~~ diffraction diameter. Therefore, it is good preferred to choose a fiber according to an the purpose of observation purpose.

[0039]

In the laser scan type fluorescence microscope according to the present invention shown by these embodiments, parallel luminous flux deflected by the scanning means (scanner section 3) is relayed to an the intermediate image position through the pupil projection lens 6.

Here, if a the pupil projection lens 6 is constituted by composed of two or more lenses, wherein a concave surface of the lens arranged nearest to the scanning-means side is directed to the scanning-means side, and a concave surface of the lens nearest to the middle-image side is directed to the middle-image side, correction of an optical performance in the intermediate image can be carried out well.

[0040]

In the laser scan type fluorescence microscope of the present invention shown by each of embodiments mentioned above, it is desirable to satisfy the following condition (2):

$$0.2 \leq F_e/D_3 \leq 0.5 \quad \dots(2)$$

where  $D_3$  is a distance from the conjugate position of conjugate with the pupil of the objective lens unit 2 and arranged near the scanning means to the intermediate image position of the image forming lens unit 5, and  $F_e$  is a focal length of the pupil projection lens 6.

[0041]

If condition (2) is satisfied, it is possible to shorten a distance from the scanning means to the intermediate image position, and to miniaturize the equipment, while keeping the optical performance of the pupil projection lens good.

If it exceeds the maximum value upper limit of the condition (2), the distance of from the scanning means and to the pupil projection lens becomes short, and an interval of the pupil projection lens and the scanning means accordingly a space between them becomes so short too much, and it generates to cause interference.

On the other hand, if less than the lower limit of the condition (2), it is difficult to miniaturize the equipment since the full length from the scanning means to the intermediate image position becomes too long too much.

[0042]

In the laser scan type fluorescence microscope shown by each of the embodiments mentioned above, which comprises two or more lens groups, having at least one cemented lens of a positive lens and a negative lens, it is desirable to satisfy the following conditions (3) and (4):

$$0.4 \leq F_{TL}/D_1 \leq 1 \quad \dots(3)$$

$$80 \leq v_p \quad \dots(4)$$

where  $v_p$  is Abbe's Number of the positive lens in this cemented lens,  $F_{TL}$  is a focal length of the image forming lens unit 5, and  $D_1$  is a distance from the position holding the objective lens unit 2 to the intermediate image position .

[0043]

If the condition (3) is satisfied, it becomes possible to shorten a distance from the position holding the objective lens shoulder to the intermediate image position, and to miniaturize the equipment. If the condition (4) is satisfied, ~~correction of~~ spherical aberration and axial chromatic aberration ~~at an axis~~ generated by shortening a shortened focal length are ~~carried out good~~ well compensated for, and a ~~co~~-focal point confocal fluorescence observation from visible region to near-infrared region can be carried out.

When a ~~co~~-focal point confocal fluorescence microscope is constituted in the present invention here, ~~none of~~ the spherical aberration and the axial chromatic aberration ~~at an axis~~ is ~~obtained appear not~~ as a picture ~~by in the~~ actually obtained picture ~~obtained in fact~~, and accordingly, but affects wave front aberration in each of wavelength and an observation domain ~~is affected~~. If the condition of wave front aberration of the optical system of the present invention is good, the picture obtained from the detector ~~becomes of~~ has high resolution and high S/N. Therefore, by correcting spherical aberration and off-axial aberration ~~off axis~~ in good condition, the wavefront aberration can be corrected good as a result. The same may be applied to each of aberrations described by the following-conditions.

If it exceeds the ~~maximum value upper limit~~ of the condition (3), an interval between the objective lens and the image forming lens becomes too short too much, and it becomes difficult to arrange a focusing mechanism ~~portion~~ by which the free working distance of the objective lens is changed.

On the other hand, if less than the lower limit of the condition (3), it becomes difficult for the full length from the sample to the scanning means to become long too much, and miniaturization of the equipment is difficult.

It is not desirable that it is less than the lower limit of the condition (4), If so, correction of the chromatic aberration at an axis and spherical aberration generated in the objective ~~lens~~ optical system becomes difficult, and accordingly the ~~co~~-focal point confocal fluorescence observation from visible region to near-infrared light of the wavelength of the excitation light, becomes difficult.

[0044]

In the laser scan type microscope of each of embodiments mentioned above, ~~if the~~ image forming lens unit 5 ~~consists of~~ includes at least two lens groups, ~~having at least that are~~ a front group at an intermediate image side, and a rear group at an objective lens side, where ~~a lens group of the front group of an image forming lens unit 5~~ has at least one negative lens, it is desirable to satisfy the following conditions (5) and (6):

$$0.4 \leq D2/FTL \leq 1$$

...(5)

$$0.7 \leq \text{FTL1}/\text{FTL} \leq 1.5$$

...(6)

where FTL1 is a focal length of the rear group of the image forming lens unit 5, and D2 is an interval of between the front group of the image forming lens unit 5 and the rear group of the image forming lens unit 5.

[0045]

Thus, if the image forming lens unit 5 is constituted by composed of two lens groups, correction of the aberration can be carried out by the rear group so that the aberration in the front group may be negated, and a suitable laser scan type fluorescence microscope can be obtained by observation *in vivo*.

If conditions (5) and (6) are satisfied, it becomes possible to correct astigmatism, coma aberration and magnification chromatic aberration better.

It is not desirable if it exceeds the maximum value upper limit of the condition (5), since the lens group of lenses in the front group becomes are located so close to the intermediate image position too much and this degrades that the image quality of the picture image by influence of blemish and garbage on is degraded by flaws or dust on a lens surface.

On the other hand, if less than the lower limit of the condition (5), chromatic aberration on the axis and coma aberration off the axis gets worse. Therefore, it is not desirable.

If it exceeds the maximum value upper limit of the condition (6), the power of the rear group becomes too weak too much, and it becomes difficult to carry out correction of color spherical aberration and coma aberration.

On the other hand, if it exceeds falls below the lower limit of the condition (6), the power of the rear group becomes too strong too much, and to achieve correction of chromatic aberration on the axis and coma aberration becomes difficult.

**On page 27, between lines 14 and 15, please insert the following new text:**

$$D = d_{24} + d_{25} + \dots + d_{44} \text{ (r}_{24} \text{ to r}_{44}\text{)}$$

$$D_1 = d_{14} + d_{15} + \dots + d_{23} \text{ (r}_{14} \text{ to r}_{24}\text{)}$$

$$D_2 = d_{18} \text{ (r}_{18} \text{ to r}_{19}\text{)}$$

$$D_3 = d_1 + d_2 + \dots + d_{13} \text{ (r}_1 \text{ to r}_{14}\text{)}$$

**On page 30, between lines 16 and 17, please insert the following new text:**

D = d<sub>24</sub> + d<sub>25</sub> + ... + d<sub>44</sub> (r<sub>24</sub> to r<sub>44</sub>)

D<sub>1</sub> = d<sub>14</sub> + d<sub>15</sub> + ... + d<sub>23</sub> (r<sub>14</sub> to r<sub>24</sub>)

D<sub>2</sub> = d<sub>18</sub> (r<sub>18</sub> to r<sub>19</sub>)

D<sub>3</sub> = d<sub>1</sub> + d<sub>2</sub> + ... + d<sub>13</sub> (r<sub>1</sub> to r<sub>14</sub>)

**On page 33, between lines 19 and 20, please insert the following new text:**

D = d<sub>25</sub> + d<sub>26</sub> + ... + d<sub>45</sub> (r<sub>25</sub> to r<sub>45</sub>)

D<sub>1</sub> = d<sub>15</sub> + d<sub>16</sub> + ... + d<sub>24</sub> (r<sub>15</sub> to r<sub>25</sub>)

D<sub>2</sub> = d<sub>19</sub> (r<sub>19</sub> to r<sub>20</sub>)

D<sub>3</sub> = d<sub>1</sub> + d<sub>2</sub> + ... + d<sub>14</sub> (r<sub>1</sub> to r<sub>15</sub>)